FORM PTO-1390

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE
TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: BE 8992

U.S. APPLN. NO. (If known, see 37 CFR 1.5): 09/786055

INTERNATIONAL APPLICATION NO.:	INTERNATIONAL FILING DATE:	PRIORITY DATE CLAIMED:
PCT/FR99/02057	27 August 1999	1 September 1998

TITLE OF INVENTION: PHOSPHOEPOXIDES, PROCESS FOR THE PRODUCTION THEREOF AND USE THEREOF

APPLICANT(S) FOR DO/EO/US: Christian BELMANT, Jean-Jacques FOURNIE, Marc BONNEVILLE and Marie-Alix PEYRAT

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [X] has been transmitted by the International Bureau. (see attached copy of PCT/IB/308)
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [X] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Item 11. to 16. below concern document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A **FIRST** preliminary amendment.
    [ ] A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information:

International Preliminary Examination Report (PCT/IPEA/409)
International Search Report (PCT/ISA/210)
Notice Informing The Applicant of the Communication of the International Application to the Designated Offices(PCT/IB/308)
Abstract of the Disclosure on a separate sheet
Application Data Sheet

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NO.
09/786055	PCT/FR99/02057	BE 8992

				CALCULATIONS PTO USE ONLY
17. [X] The following fees are submitted:				
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):				
Neither international preliminary examination fee (37 CFR1.482) nor international search fee (37 CFR1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... $ 1,000.00				
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... $ 860.00				
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... $ 710.00				
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... $ 690.00				
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) ..... $ 100.00				
			ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00
Surcharge of $130.00 for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492(e)).				$
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	$
Total claims	28 - 20 =	8	X $18.00	$ 144.00
Independent claims	6 - 3 =	3	X $80.00	$ 240.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $270.00	$
			TOTAL OF ABOVE CALCULATIONS =	$ 1,244.00
Reduction of ½ for filing by small entity, if applicable. Applicant claims Small Entity Status under 37 CFR 1.27.			+	$
			SUBTOTAL =	$ 1,244.00
Processing fee of $130 for furnishing the English translation later than months from the earliest claimed priority date (37 CFR1.49(f)).			+	$
			TOTAL NATIONAL FEE =	$ 1,244.00
Fee for recording the enclosed assignment (37 CFR1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property			+	$
			TOTAL FEES ENCLOSED =	$ 1,244.00
			Amount to be refunded:	
			charged:	

a. [X] A check in the amount of $ **1,244.00** to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. **25-0120** in the amount of $ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required by 37 CFR 1.16 and 1.17, or credit any overpayment to Deposit Account No. **25-0120**. A duplicate copy of this sheet is enclosed.

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March 1, 2001

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APPLICATION INFORMATION

Title Line One::	PHOSPHOEPOXIDES, PROCESS FOR THE
Title Line Two::	PRODUCTION THEREOF AND USE THEREOF
Total Drawing Sheets::	2
Formal Drawings?::	YES

Application Type::	UTILITY
Docket Number::	BE 8992

REPRESENTATIVE INFORMATION

Representative Customer Number::	000466

CONTINUITY INFORMATION

This application is a::	371 OF
>Application One::	PCT/FR99/02057
Filing Date::	27 AUGUST 1999

PRIOR FOREIGN APPLICATION

Foreign Application One::	98.10914
Filing Date::	1 SEPTEMBER 1998
Country::	FRANCE
Priority Claimed::	YES

PHOSPHOEPOXIDES, PROCESS FOR THE PRODUCTION THEREOF AND USE THEREOF.

This invention relates to novel phosphoepoxides, to the process for the production thereof and to the use thereof for stimulating Tγ9δ2 lymphocytes bearing TCR receptors with Vγ9 and Vδ2 variable regions.

In healthy individuals, the Tγδ lymphocytes of primates (humans, monkeys) present in the peripheral bloodstream usually constitute from 1 to 5% of the lymphocytes in the blood and play a role in the immune system. It has been demonstrated that they recognize their antigenic ligands by direct interaction with the antigen without presentation by molecules of the MHC by a presenting cell. Tγ9δ2 lymphocytes (sometimes also known as Tγ2δ2 lymphocytes) are Tγδ lymphocytes bearing TCR receptors with Vγ9 and Vδ2 variable regions. They constitute the majority of Tγδ lymphocytes in human blood.

When activated, Tγδ lymphocytes exercise a strong cytotoxic activity which is unrestrained by the MHC and is particularly effective in killing various types of cells, in particular pathogenic cells. These may be cells infected by viruses ("γδ T cell activation or anergy during infections: the role of nonpeptidic TCR ligands and HLA class I molecules" Fabrizio POCCIA *et al*, Journal of Leukocyte Biology, 62, 1997, p. 1-5), or by other intracellular parasites, such as mycobacteria ("The antituberculous *Mycobacterium bovis* BCG Vaccine is an attenuated Mycobacterial producer of phosphorylated nonpeptidic Antigens for human γδ T cells" Patricia CONSTANT *et al*, Infection and Immunity, vol. 63, no. 12, Dec. 1995, p. 4628-4633); or by protozoans (*"Plasmodium falciparum* stimuli for human γδ T Cells are related to phosphorylated Antigens of mycobacteria" Charlotte BEHR *et al*, Infection and Immunity, Vol. 64, no. 8, 1996, p. 2892-2896). They may also be cancer cells ("CD94/NKG2 inhibitory receptor complex modulates both antiviral and antitumoral responses of polyclonal phosphoantigen-reactive Vγ9 Vδ2 T lymphocytes" Fabrizio POCCIA *et al*, Journal of Immunology, 159, p. 6009-6015; "Stimulation of γδ T cells by phosphoantigens" Jean-Jacques FOURNIE, Marc BONNEVILLE, Res. Immunol., 66th FORUM IN IMMUNOLOGY, 147, p. 338-347).

It has been demonstrated that, in the event of a mycobacterial infection, human Tγ9δ2 lymphocytes react to four natural, nonpeptidic molecules of a phosphorylated structure, known as phosphoantigens, which exhibit stimulation activity at a concentration of 1 to 5 nM (nanomolar) (WO-95/20673 and "Stimulation of human γ δ T cells by nonpeptidic Mycobacterial ligands" Patricia CONSTANT *et al*, Science, 264, p. 267-270).

These natural antigens have not been completely identified. Certain authors have erroneously presented them as alkene derivatives of pyrophosphate, in particular isopentenyl pyrophosphate IPP (US-5 639 653 and "Natural and Synthetic nonpeptide antigens recognized by human γδ T cells", Yoshimasa TANAKA *et al,* Nature, 375, 1995, p. 155-158). It has nonetheless now been demonstrated that none of these prenyl pyrophosphates is active at a concentration of nanomolar magnitude. The best results which have been obtained have been unable to demonstrate activity at below 3 μM for IPP and below 0.3 μM for dimethylallyl-UTP and 3-methyl-2-hexene pyrophosphate. The minimum active concentration of these compounds is thus, at best, of the order to 100 times higher than that of natural phosphoantigens.

With regard to IPP, it should in particular be noted that the most recent of the above-stated publications make the mistake of deducing the structure of the isopentenyl radical solely on the basis of mass spectrometry and the detection of a certain level of bioactivity. Indeed, apart from the fact that the compound analyzed in these publications was not purified and that a mass spectrum cannot identify uncharged species, it may be demonstrated that there are in fact several thousand different chemical structures which may have the same molecular weight and be a substituent of pyrophosphate in these molecules.

The fact that the minimum active concentration for IPP is much higher (some 1000 times higher) and that the intensity of the Tγ9δ2 lymphocyte responses obtained is much weaker than that for natural phosphoantigens demonstrates that IPP is not one of these natural phosphoantigens ("A novel nucleotide-containing antigen for human blood γδ T lymphocytes", Y. Poquet *et al,* Eur. J. Immunol. 1996, 26, p. 2344-2349). This is moreover confirmed by numerous other observations: IPP is not found in sufficient concentration in mycobacterial extracts which stimulate Tγ9δ2 lymphocytes; according to "High pH anion exchange chromatographic analysis of

phosphorylated compounds: application to isolation and characterization of non peptide mycobacterial antigens", Y. Poquet *et al*, Anal. Biochem, 243 no. 1, 1996, p. 119-126, IPP does not have the same chromatographic (HPAEC) characteristics as natural phosphoantigens; IPP and other natural isoprenoids are produced by all living cells, but these do not stimulate Tγ9δ2 lymphocytes.

Moreover, it is known that substances having bioactivity of the order of or greater than 1 μM are only rarely compatible with the economic constraints of operation on an industrial scale. The synthetic phosphoantigens which have hitherto been proposed thus cannot be processed on an industrial scale under acceptable economic conditions.

Natural phosphoantigens, on the other hand, may only be produced in very small quantities (WO 95/20673) and, since their precise chemical structure has still not yet been established, they cannot be synthesized. Economic industrial scale processing is thus likewise out of the question, despite their demonstrated great therapeutic worth.

The object of the invention is accordingly to provide novel chemical compounds which activate Tγ9δ2 lymphocytes at a minimum activation concentration of below 100 nM, in particular of the order of 10 nM.

A further object of the invention is to provide compounds which may be linked to a large number of organic groups, in particular to natural or synthetic peptide groups, so as to permit multifunctional compounds to be obtained.

A further object of the invention is to provide a process for the production of the compounds according to the invention.

A further object of the invention is to suggest uses according to the invention as Tγ9δ2 lymphocyte activators and in particular therapeutic uses of the compounds according to the invention.

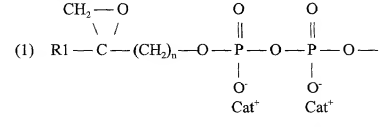
A further object of the invention is to provide such compounds which may be synthesized simply, quantitatively and at low cost, i.e. in a manner compatible with the economic constraints of production on an industrial scale.

A further object of the invention is to provide an advantageous synthetic pathway for these compounds.

A further object of the invention is to provide a process for the production of the compounds according to the invention.

A further object of the invention is to suggest uses for the compounds according to the invention as a Tγ9δ2 lymphocyte activator and in particular therapeutic uses of the compounds according to the invention.

The invention accordingly provides compounds comprising at least one phosphoepoxide group of the formula:



(1)

where R1 is selected from among —CH_3 and —CH_2—CH_3,

Cat^+ represents one or more organic or inorganic cation(s) (including the proton), which may be identical or different in the same compound,

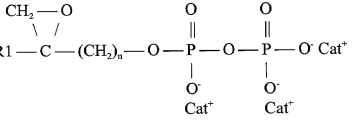
and n is an integer between 2 and 20.

A compound according to the invention may in particular comprise one or more epoxide group(s) selected from among the esters of the following groups (IUPAC nomenclature) or among the compounds formed from these groups: 3,4-epoxy-3-methyl-1-butyl diphosphate; 3,4-epoxy-3-ethyl-1-butyl diphosphate; 4,5-epoxy-4-methyl-1-pentyl diphosphate; 4,5-epoxy-4-ethyl-1-pentyl diphosphate; 5,6-epoxy-5-methyl-1-hexyl diphosphate; 5,6-epoxy-5-ethyl-1-hexyl diphosphate; 6,7-epoxy-6-methyl-1-heptyl diphosphate; 6,7-epoxy-6-ethyl-1-heptyl diphosphate; 7,8-epoxy-7-methyl-1-octyl diphosphate; 7,8-epoxy-7-ethyl-1-octyl diphosphate; 8,9-epoxy-8-methyl-1-nonyl diphosphate; 8,9-epoxy-8-ethyl-1-nonyl diphosphate; 9,10-epoxy-9-methyl-1-decyl diphosphate; 9,10-epoxy-9-ethyl-1-decyl diphosphate; 10,11-epoxy-10-methyl-1-undecyl diphosphate; 10,11-epoxy-10-ethyl-1-undecyl diphosphate; 11,12-epoxy-11-methyl-1-dodecyl-diphosphate; 11,12-epoxy-11-ethyl-1-dodecyl diphosphate; 12,13-epoxy-12-methyl-1-tridecyl diphosphate; 12,13-epoxy-12-ethyl-1-tridecyl diphosphate; 13,14-epoxy-13-methyl-1-tetradecyl diphosphate; 13,14-epoxy-13-ethyl-1-tetradecyl diphosphate; 14,15-epoxy-14-methyl-1-pentadecyl diphosphate; 14,15-epoxy-14-ethyl-1-pentadecyl diphosphate; 15,16-epoxy-15-methyl-1-

hexadecyl diphosphate; 15,16-epoxy-15-methyl-1-hexadecyl diphosphate; 16,17-epoxy-16-methyl-1-heptadecyl-diphosphate; 16,17-epoxy-16-ethyl-1-heptadecyl diphosphate; 17,18-epoxy-17-methyl-1-octadecyl diphosphate; 17,18-epoxy-17-ethyl-1-octadecyl diphosphate; 18,19-epoxy-18-methyl-1-nonadecyl diphosphate; 18,19-epoxy-18-ethyl-1-nonadecyl diphosphate; 19,20-epoxy-19-methyl-1-eicosyl diphosphate; 19,20-epoxy-19-ethyl-1-eicosyl diphosphate; 20,21-epoxy-20-methyl-1-heneicosyl diphosphate; 20,21-epoxy-20-ethyl-1-heneicosyl diphosphate; 21,22-epoxy-21-methyl-1-docosyl diphosphate; 21,22-epoxy-21-methyl-1-docosyl diphosphate.

The invention also in particular relates to the novel phosphoepoxides of the following formula:

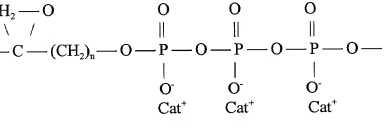
(2)



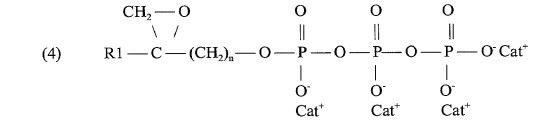
for the use thereof as therapeutically active substances. It should be noted that the publication by M. MUEHLBACHER *et al*, "Isopentenyl-diphosphate isomerase: inactivation of the enzyme with active-site-directed irreversible inhibitors and transition-state analogs", Biochemistry, vol. 27, no. 19, p. 7315-7328 (1988) has already described a compound according to the formula (2) in which R1 is CH3 and n = 2, as well as the *in vitro* action thereof as an enzyme inhibitor on isopentenyl pyrophosphate isomerase purified from the mould *Claviceps purpurea*. Said document discloses no therapeutic use of this compound.

The invention accordingly also provides novel compounds comprising at least one phosphoepoxide group of the formula:

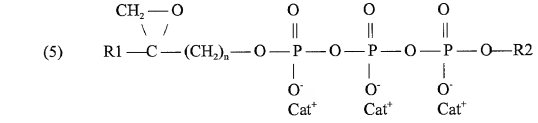
(3)



Among the novel compounds according to the invention of the formula (3) phosphoepoxide compounds which may be mentioned are those of the formula:

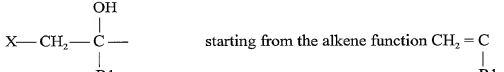


and the phosphoepoxide compounds of the formula:



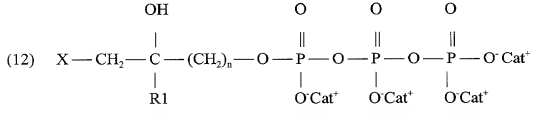
where R2 is an organic or inorganic substituent selected from among the group comprising:

- substituents which do not prevent formation of the halohydrin function

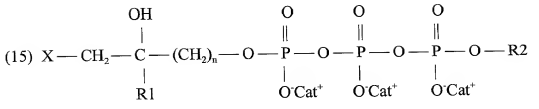


and halogen X_2 in the presence of water;

- and substituents for which there is an R2-O-Y compound which is not reactive towards the halohydrin function of the compound of the formula:



and selected such that R2-O-Y may react with the terminal phosphate of this compound (12) in order to obtain the compound (15):



- and substituents for which there is a compound R2-O-PPP, where PPP denotes the triphosphate group.

Said compounds according to the invention are advantageously wherein n = 2 and R1 is CH_3.

The compounds according to the invention advantageously additionally comprise at least one group selected from among the group comprising nucleoside derivatives, oligonucleotides, nucleic acids (RNA, DNA), amino acids, peptides, proteins, monosaccharides, oligosaccharides, polysaccharides, fatty acids, simple lipids, complex lipids, folic acid, tetrahydrofolic acid, phosphoric acids, inositol, vitamins, co-enzymes, flavonoids, aldehydes, halohydrins and epoxides.

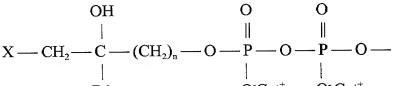
In particular, the invention provides the phosphoepoxide compounds of the formula (5) above in which R2 is selected from among the group comprising nucleoside derivatives, oligonucleotides, nucleic acids (RNA, DNA), amino acids, peptides, proteins, monosaccharides, oligosaccharides, polysaccharides, fatty acids, simple lipids, complex lipids, folic acid, tetrahydrofolic acid, phosphoric acids, inositol, vitamins, co-enzymes, flavonoids, aldehydes, halohydrins, phosphoepoxides of the formula (1) and epoxides.

The invention also provides compounds, the structure of which incorporates two or more groups of the formula (1), which may be identical or different, for example monomers, polymers, oligomers or dendrimers, or more generally molecules with two or more phosphorylated branches of the formula (1).

It should be noted that the compounds according to the invention are esters (monoesters or diesters) of phosphoric acid (this term encompassing those acids in which phosphorus is in oxidation state V, namely orthophosphoric acid, pyrophosphoric acid, metaphosphoric acid, triphosphoric acid, other polyphosphoric acids).

The invention provides a process for the production of the compounds according to the invention. This process is characterized in that:

- an intermediate compound comprising at least one phosphohalohydrin group of the formula is first prepared:

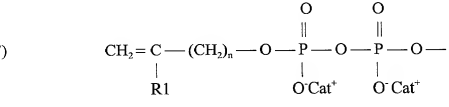


(6)

where X is a halogen selected from among iodine, bromine and chlorine,

- the intermediate compound is reacted with a hydroxide-producing medium in order to convert the halohydrin functions of the intermediate compound into epoxide functions.

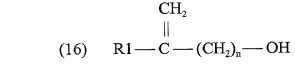
Advantageously and according to the invention, in order to prepare said intermediate compound, the halogen X_2 is reacted in the presence of water with a starting compound comprising at least one phosphorylated alkene group of the formula:



(7)

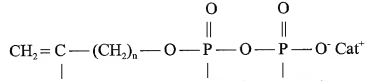
Advantageously and according to the invention, a salt formed from said starting compound is reacted in an aqueous or aqueous/alcoholic medium, at neutral pH, at a temperature of below 30°C, by mixing with an aqueous solution of the halogen X_2. Advantageously and according to the invention, the reaction is performed at atmospheric temperature at a temperature of between 0°C and 25°C.

The starting compounds may themselves be obtained from alcohol:

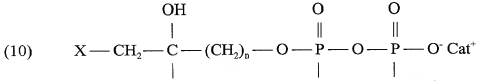


(16)

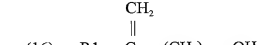
Advantageously and according to the invention, the starting compound is a salt of the formula:

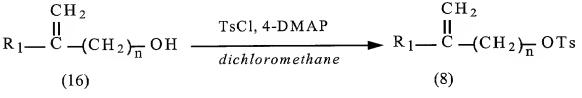
(9) 

The intermediate phosphohalohydrin compound of the following formula is then obtained:

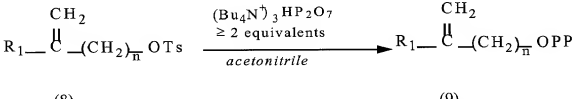
(10) 

An example of a complete reaction scheme for obtaining the intermediate compound (10) from the alcohol (16) is given below:

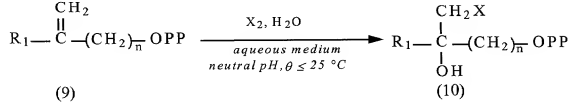
(16) 



(16) (8)



(8) (9)



(9) (10)

where TsCl is tosyl chloride,

4-DMAP is 4-dimethylaminopyridine,

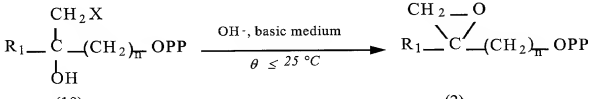
Bu_4N+ is tetrabutylammonium,

$(Bu_4N+)_3HP_2O_7$ is tris(tetra n-butylammonium) hydrogenpyrophosphate,

PP represents the pyrophosphate group.

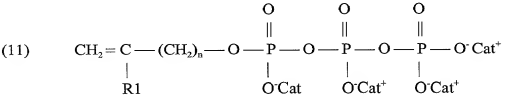
The reactions which allow compound (9) to be obtained from the alcohol (16) may be performed as described by: DAVISSON V.J. *et al.* "Phosphorylation of Isoprenoid Alcohols" J. Org. Chem 1986, 51, 4768-4779, and DAVISSON V.J. *et al.* "Synthesis of Allylic and Homoallylic Isoprenoid Pyrophosphates" Methods in Enzymology, 1984, 110, 130-144.

Starting from the intermediate compound (10), the compound of the formula (2) according to the invention is obtained in accordance with the following scheme:

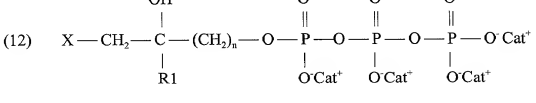


(10) (2)

Advantageously and according to the invention, the starting compound is a salt of the formula:

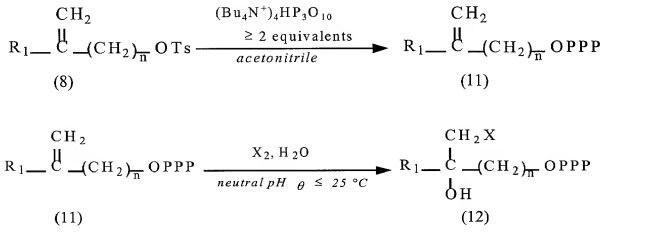


The triphosphohalohydrin compound of the following formula is then obtained:



The triphosphoepoxide compound (4) according to the invention is then obtained.

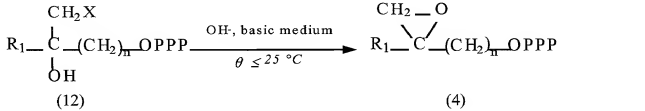
An example of a complete reaction scheme for obtaining the compound (4) according to the invention from the alcohol (16) is given below:



where PPP is the triphosphate group,

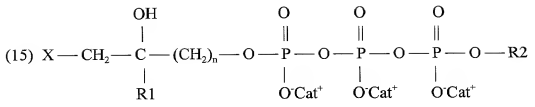
$(Bu_4N^+)_4HP_3O_{10}$ is tetrakis(tetra-n-butylammonium) hydrogentriphosphate. The compound (8) is obtained from the alcohol (16) as stated above.

Starting from the intermediate compound (12), the triphosphoepoxide compound according to the invention of the formula (4) is obtained in accordance with the following scheme:

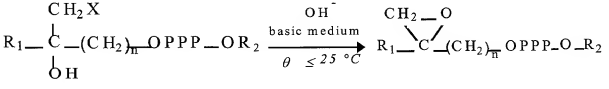


Several variants are possible for preparing a phosphoepoxide compound according to the invention of the formula (5).

In a first variant, a phosphohalohydrin intermediate compound of the following formula is first prepared:

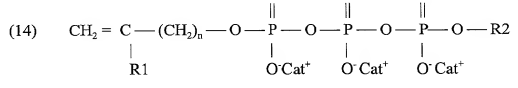


The compound (5) is thus obtained in accordance with the following reaction:

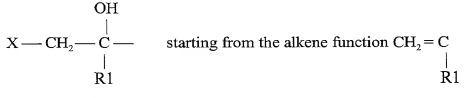


(15) (5)

To this end, the process may be performed as stated above (reaction of X_2 in the presence of water with a phosphorylated alkene function) by using a salt of the following formula as the starting compound:



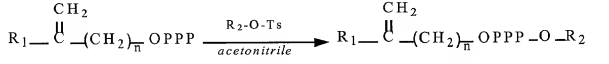
where R2 is an organic or inorganic substituent of a nature such as not to prevent formation of the halohydrin function



and halogen X_2 in the presence of water.

The starting compound (14) may itself be prepared in accordance with one of the following reaction schemes:

- Reaction scheme 1:

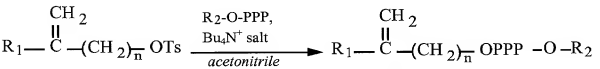


(11) (14)

where Ts is tosyl.

The compound (11) may be obtained as stated above starting from the alcohol (16) and the intermediate compound (8). The reaction which allows compound (14) to be obtained from the compound (11) may be performed under conditions similar to those described in the publications by DAVISSON V.J. *et al.* This scheme may be used when R2-O-Ts is commercially available.

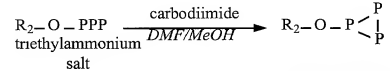
- Reaction scheme 2:



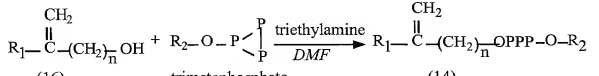
(8) (14)

The intermediate compound (8) may be obtained as stated above starting from the alcohol (16). The reaction which allows compound (14) to be obtained from the compound (8) may be performed under conditions similar to those described in the publications by DAVISSON V.J. *et al.* This scheme may be used when R2-O-PPP is commercially available.

- Reaction scheme 3:



trimetaphosphate



(16) trimetaphosphate (14)

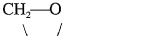
where DMF is dimethylformamide,

MeOH is methanol.

This reaction scheme 3 may be performed under conditions similar to those described in D.G. KNORRE *et al* "General method for the synthesis of ATP gamma derivatives" Febs letters, 1976, 70, 105-108.

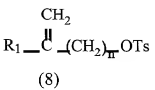
This reaction scheme 3 cannot be used when R2 comprises a carbodiimide-reactive function (carboxylate, triphosphate etc.). It is, however, advantageous when R2-O-PPP is commercially available.

In the specific case where R2- is itself an epoxide group of the formula:

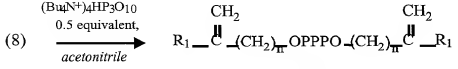


(18)

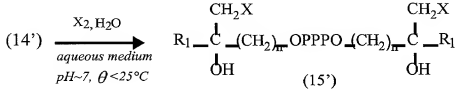
the following reaction scheme may be used:



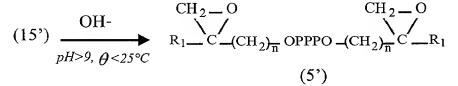
(8)



(14')



(15')

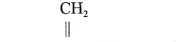


(5')

The compound (5') is a particular instance of the compound (5) according to the invention.

It should be noted that, in all these reactions, acetonitrile may be replaced by any other aprotic dipolar solvent (dimethylformamide DMF, dimethyl sulfoxide DMSO etc.).

It should be noted that when preparing compounds (2), (4) and (5') and in the event that $n \neq 2$, the intermediate compound (8) may also be replaced by the chloride or bromide compound of the formula:



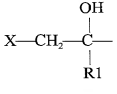
(19) $R1-C(=CH_2)-(CH_2)_n-A$

where A is chlorine or bromine.

The alcohols (16) are commercially available or may readily be obtained by a well known Grignard reaction between an alkenyl organomagnesium compound and formaldehyde or ethylene oxide.

In a second variant, a compound of the formula (15) could be prepared by reacting the intermediate triphosphohalohydrin compound of the formula (12) starting from a salt soluble in an organic medium, such as a Bu_4N+ salt, in a subsequent stage with a compound R2-O-Y, where -O-Y is a leaving group and R2 is an organic or inorganic substituent selected such that R2-O-Y is capable of forming, by reaction with the compound (12), the intermediate compound of the formula (15).

In order to be capable of forming the intermediate compound of the formula (15) in this manner, the compound R2-O-Y must in particular not be reactive towards the halohydrin function:



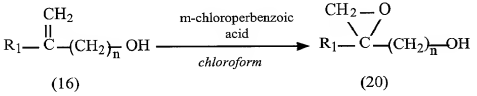
Moreover, R2-O-Y must react with the terminal phosphate of compound (12) to form the compound (15).

The reaction of the compound of the formula (12) with R2-O-Y is a nucleophilic substitution. This reaction is in particular possible and advantageous for R2 selected from among the group comprising alkyls and alkenes. Y is selected such that R2-O-Y may give rise to the compound (15) by nucleophilic substitution. Y is selected, for example, from among tosyl, brosyl and triflyl.

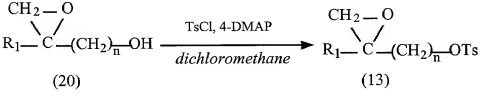
This second variant then permits the phosphoepoxide compound according to the invention of the formula (5) to be prepared by reacting the intermediate

compound (15) in a basic aqueous medium in order to convert the halohydrin functions of the intermediate compound (15) into epoxide functions as stated above.

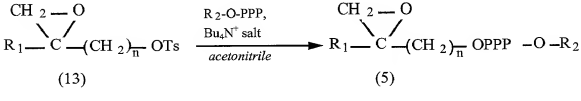
In a third variant, the phosphoepoxide according to the invention may be prepared, without passing via the intermediate compound (15), starting from the alcohol (16) in accordance with the following reaction scheme:



(16) (20)



(20) (13)



(13) (5)

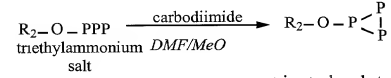
This third variant is in particular advantageous when R2-O-PPP is commercially available or when it is not possible to prepare the phosphohalohydrin intermediate compound (15).

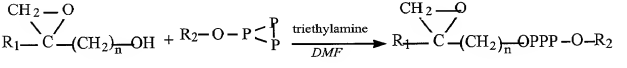
The first stage of this reaction which involves converting the alkene function into an epoxide function may be performed as stated by M. MUEHLBACHER *et al.* "Isopentenyl-diphosphate isomerase: inactivation of the enzyme with active-site-directed irreversible inhibitors and transition-state analogs", Biochemistry, vol. 27, no. 19, p 7315-7328 (1988).

This reaction scheme may also be used directly to obtain the monoester compounds (2) and (4) according to the invention. Nevertheless, with these

monoester compounds, production starting from phosphohalohydrin intermediate compounds is generally faster, more quantitative and easier to perform.

In a fourth variant, the following reaction scheme is used:





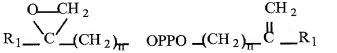
(20) trimetaphosphate (5)

This reaction may be performed under the conditions described by D.G. KNORRE *et al* "General method for the synthesis of ATP gamma derivatives" Febs letters, 1976, 70, 105-108.

A compound according to the invention may accordingly be difunctional or polyfunctional. The phosphoepoxide function(s) bring(s) about a specific desired antigenic property towards Tγ9δ2 lymphocytes, and R2 or the other functional groups of the compound may exhibit other, in particular therapeutic, properties.

In the case of a compound according to the invention having two or more phosphoepoxide groups of the formula (1), it is sufficient to start from a starting compound having the corresponding number of phosphorylated alkene groups of the formula (7) and the corresponding chemical structure, or to use the compound of the formula (12) and to react it with an intermediate compound R2-O-Y having the corresponding number of -O-Y functions (second variant), or to use a compound R2-O-PPP which already contains other epoxide functions (third and fourth variants).

The invention also in particular relates to the novel phosphoepoxide β ester compounds of the formula:

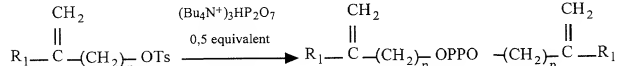


(21)

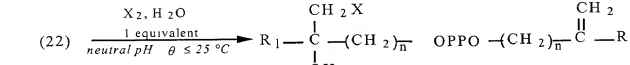
where R1 is selected from among $—CH_3$ and $—CH_2—CH_3$,

n is an integer between 2 and 20.

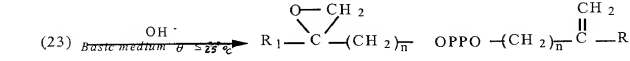
These compounds may be obtained in accordance with the following process:



(8) (22)



(23)



(21)

The invention also provides uses of the compounds according to the invention as primate Tγ9δ2 lymphocyte activators, in particular to activate proliferation and/or cytotoxic activity and/or production of mediator substance(s) for primate Tγ9δ2 lymphocytes with TCR receptors comprising Vγ9 and Vδ2 variable regions.

The invention also provides uses of the compounds according to the invention for the treatment of cells sensitive to primate Tγ9δ2 lymphocytes in a natural or artificial medium which may contain Tγ9δ2 lymphocytes, in which medium said cells may be brought into contact with Tγ9δ2 lymphocytes and which is compatible with the compounds according to the invention (i.e. which is not likely to cause the breakdown thereof, at least under certain treatment conditions).

A "cell sensitive to Tγ9δ2 lymphocytes" is taken to mean any cell subject to the induced effector activity of the Tγ9δ2 lymphocytes: cell death (cell

destruction by Tγ9δ2 lymphocytes); reception of cytokine released by Tγ9δ2 lymphocytes (TNF-α, INF-γ...); possibly cellular proliferation induced by Tγ9δ2 lymphocytes.

The invention accordingly provides a Tγ9δ2 lymphocyte activation process, in particular a process for activating the proliferation of Tγ9δ2 lymphocytes and/or the cytotoxic activity of Tγ9δ2 lymphocytes and/or the production of mediator substance(s) by Tγ9δ2 lymphocytes, in which process these Tγ9δ2 lymphocytes are brought into contact with at least one compound according to the invention in a medium which contains Tγ9δ2 lymphocytes and is compatible with T lymphocyte growth. Advantageously and according to the invention, interleukin, in particular interleukin-2 (IL-2), is introduced into the medium in a proportion suitable to bring about lymphocyte growth in this medium. Indeed, the presence of the lymphocyte growth factor IL-2 is essential to achieve proliferation of the T lymphocytes, among which only the Tγ9δ2 lymphocytes have been activated by a compound according to the invention. This growth factor must thus be present in the medium for those uses in which proliferation of Tγ9δ2 lymphocytes is desired. This lymphocyte growth factor may pre-exist in the natural state or be induced or introduced into the medium, simultaneously or not with the incorporation of the compound according to the invention, in the same therapeutic composition or not. Nevertheless, in certain uses in which activation without proliferation of the Tγ9δ2 lymphocytes is desired (for example induced cytotoxicity), the presence of this growth factor is not helpful.

More specifically, the invention provides uses of the compounds according to the invention for therapeutic purposes for the curative or preventive treatment of pathological conditions producing cells sensitive to primate Tγ9δ2 lymphocytes in a medium which may contain these Tγ9δ2 lymphocytes and in which these cells may be brought into contact with the Tγ9δ2 lymphocytes.

Advantageously and according to the invention, at least one compound according to the invention is used at a concentration in the medium which brings about activation of polyclonal proliferation of Tγ9δ2 lymphocytes. This medium may be selected from among human blood, non-human primate blood, human blood extracts, non-human primate blood extracts.

Said medium may be extracorporeal, said activation process according to the invention then being an extracorporeal cellular treatment, in particular applicable in a laboratory, for studying Tγ9δ2 lymphocytes or the properties thereof, or for diagnostic purposes. The invention also provides a composition for extracorporeal (*ex vivo*) diagnostics, wherein it comprises at least one compound according to the invention.

Said medium may also be intracorporeal, activation of the Tγ9δ2 lymphocytes then being of therapeutic use.

More particularly, said medium is the peripheral bloodstream of a primate. The invention accordingly in particular provides a process for the activation of Tγ9δ2 lymphocytes in the peripheral bloodstream of a primate, in particular humans, into which is administered at least one compound according to the invention in a quantity suitable for activating Tγ9δ2 lymphocytes. At least one compound according to the invention is thus administered by a general route, in particular parenterally into the peripheral bloodstream.

Said medium may also comprise a cellular site to be treated and at least one compound according to the invention is administered directly in contact with the cellular site to be treated (topical administration).

The invention accordingly in particular provides therapeutic uses of the compounds according to the invention for treating pathological conditions in primates, said conditions belonging to the group comprising cancers, infectious diseases, in particular mycobacterial infections (leprosy, tuberculosis etc.); parasitic conditions (malaria etc.); pathological immunodeficiency syndromes (AIDS etc.). According to the invention, a therapeutic composition is administered which is suitable for releasing into the peripheral bloodstream and/or at a cellular site to be treated a quantity of at least one compound according to the invention capable of activating Tγ9δ2 lymphocytes. Indeed, it has been demonstrated in general terms in the above-stated prior art that a composition having the property of activating Tγ9δ2 lymphocytes may advantageously be used for treating these pathological conditions.

As is conventional, throughout the text the terms "therapy" or "therapeutic" encompass not only curative treatment or care, but also preventive treatment (prophylaxis), such as vaccination, together with intracorporeal diagnostics (administration for diagnostic purposes). Indeed, by permitting activation of Tγ9δ2

lymphocytes, the invention allows immunostimulatory treatments which may be advantageous not only prophylactically by preventing the development of pathogenic cells sensitive to Tγ9δ2 lymphocytes, but also curatively by inducing destruction of pathogenic cells sensitive to Tγ9δ2 lymphocytes.

The invention accordingly provides a therapeutic composition comprising at least one compound according to the invention. More specifically, the invention relates to a therapeutic composition comprising a quantity capable of being administered to a primate, in particular in contact with the peripheral bloodstream or topically, of at least one compound according to the invention, in particular for the preventive or curative treatment of the above-stated pathological conditions. A composition according to the invention may be an immunostimulant composition or a vaccine, the compounds according to the invention being antigens which activate Tγ9δ2 lymphocytes.

Advantageously and according to the invention, the therapeutic composition is characterized in that it moreover comprises a proportion of interleukin, in particular interleukin-2, suitable for bringing about lymphocyte growth in the medium into which it is to be administered.

A therapeutic composition according to the invention may be prepared in a dosage form capable of being administered by a general route, in particular parenterally directly into the peripheral bloodstream of a primate, with at least one compound according to the invention in a quantity suitable to activate Tγ9δ2 lymphocytes and one or more appropriate excipient(s). Given the very low active concentration of the compounds according to the invention (of the order of 1 to 100 nM), such administration may be made without risk of toxicity.

A therapeutic composition according to the invention may also be prepared in a dosage form appropriate for topical administration, directly in contact with the cells sensitive to Tγ9δ2 lymphocytes.

The dosage form of a therapeutic composition according to the invention is produced in accordance with the selected route of administration using conventional pharmaceutical formulation methods. The quantity and concentration of compound(s) according to the invention and the dosage are determined by reference to known chemotherapeutic methods for the diseases to be treated, taking account of the

bioactivity of the compounds according to the invention towards Tγ9δ2 lymphocytes, the individual to be treated, the disease in question and the desired biological effects.

Advantageously and according to the invention, in the case of a bioactive compound at a concentration of between 10 nM and 100 nM, the quantity of compound(s) according to the invention administered by a general route is between 1 µg and 1000 µg, in particular between 10 µg and 100 µg, per kilogram of patient body weight.

It has moreover been demonstrated *in vitro* that the compounds according to the invention exhibit no general toxicity even at concentrations of up to 100 µM, i.e. of the order of 10^4 times the bioactive concentration. Furthermore, it is known that the biochemical class of molecules to which the compounds according to the invention belong (phosphoesters) comprises a family of metabolic compounds found in any living cell. The compounds according to the invention thus exhibit no toxic effects other than those induced by the bioactivity thereof upon Tγ9δ2 lymphocytes.

Moreover, certain compounds according to the invention have a sufficiently low molecular weight (in particular below 500) to be compatible with the elimination thereof via the kidneys and urine.

One example formulation of an injectable therapeutic composition according to the invention for a primate weighing 1 kg is as follows: 50 µg of 3,4-epoxy-3-methyl-1-butyl diphosphate (Epox-PP) diluted in 0.5 ml of sterile phosphate buffer at pH 7 and adjusted to 37°C.

In this manner, 50 µg of Epox-PP (compound of the formula (2)) are administered per 1 kg of animal body weight, corresponding to a concentration in the circulating blood such as to be greater than the bioactive concentration of Epox-PP (a concentration of 100 nM of Epox-PP corresponding to approx. 50 ng/ml).

It should be noted that the excipients or other conventionally used pharmaceutically acceptable additives are chemically compatible with the compounds according to the invention.

A therapeutic composition according to the invention may also advantageously comprise one or more other active ingredient(s), in particular to bring about a synergistic effect. In particular, a compound according to the invention may act as a vaccine adjuvant. The therapeutic vaccine composition according to the invention

then comprises a known vaccine composition to which is added a quantity of compound(s) according to the invention capable of activating the Tγ9δ2 lymphocytes which will not only be able to exert their anti-infective activity directly, but will also be able to activate the T lymphocytes which effect the conventional vaccine response.

A therapeutic composition according to the invention may also itself incorporate primate Tγ9δ2 lymphocytes in a culture in a medium compatible with T lymphocyte growth. It may then be used for treating primates, or more generally vertebrates with which administration of primate Tγ9δ2 lymphocytes may be performed under conditions of immune compatibility towards said primate Tγ9δ2 lymphocytes. Such a composition according to the invention may be administered by a general route, or even by a topical route, in contact with target pathogenic cells, sensitive to said primate Tγ9δ2 lymphocytes.

The invention also provides the use of at least one compound according to the invention for the production of a therapeutic composition according to the invention. More particularly, the invention relates to the use of at least one compound according to the invention for the production of a therapeutic composition intended for the preventive or curative treatment of a pathological condition of humans or vertebrates which produces cells sensitive to primate Tγ9δ2 lymphocytes, in particular a pathological condition selected from the group comprising cancers, infectious diseases, parasitic conditions and pathological immunodeficiency syndromes. To this end, the invention also provides the use of at least one compound according to the invention for the production of a therapeutic composition intended to be administered, in particular in contact with the peripheral bloodstream or by a topical route, to a primate, in particular to humans, for the preventive or curative treatment of a pathological condition as mentioned above.

The invention also provides a process for the production of a composition, in particular a therapeutic composition, according to the invention having the characteristic of activating Tγ9δ2 lymphocytes, in which process at least one compound according to the invention is used.

The invention also relates to a process for the production of a therapeutic composition intended for the preventive or curative treatment of a pathological condition of humans or vertebrates which produces cells sensitive to primate

Tγ9δ2 lymphocytes, in which process at least one compound according to the invention is used. The invention in particular relates to a process for the production of a therapeutic composition intended to be administered, in particular in contact with the peripheral bloodstream or by a topical route, to a primate for the preventive or curative treatment of a pathological condition which produces cells sensitive to Tγ9δ2 lymphocytes, in particular a pathological condition belonging to the group stated above, in which process at least one compound according to the invention is used.

Advantageously and according to the invention, in a production process according to the invention, at least one compound according to the invention is brought into contact with a medium which contains primate Tγ9δ2 lymphocytes, and is compatible with T lymphocyte growth, in a quantity suitable to activate these Tγ9δ2 lymphocytes in this medium. Advantageously and according to the invention, said medium comprises a substance selected from among primate blood and primate blood extracts. A therapeutic composition containing activated Tγ9δ2 lymphocytes is then obtained so allowing a cellular therapeutic approach to be performed.

It should be noted that the compounds according to the invention are epoxide compounds and do not correspond to natural phosphoantigens, in particular to the molecules known as Tubag1, Tubag2, Tubag3 and Tubag4 obtained as described in WO 95/20673. It is in any event possible to demonstrate for example that these natural phosphoantigens are broken down by bromine water or by treatment with sodium borohydride in a basic aqueous medium, whereas the compounds according to the invention are insensitive to these reagents. The compounds according to the invention are thus not natural antigens, but are synthetic antigens which activate Tγ9δ2 lymphocytes at concentrations of the same order and with an efficiency similar to or even greater than that of natural antigens.

It should also be noted that, contrary to the prior art as illustrated by US 5,639,653, which considered that the presence of an alkyl or alkene group was essential to activate human Tγ9δ2 lymphocytes, the inventors have observed that by destroying the alkene bond and replacing it with an epoxide group, the Tγ9δ2 lymphocytes are activated extremely strongly and at very low concentration. In particular, it may be observed that the effect may even exceed that of phosphoantigens of natural origin.

Further features, objects and advantages of the invention will be evident from the following Examples, which are provided in non-limitative manner merely for purposes of explanation, and from the figures:

- Figure 1 is a graph showing the results obtained in Example 5,
- Figure 2 is a graph showing the results obtained in Example 6.

EXAMPLE 1: Production of 3,4-epoxy-3-methyl-1-butyl diphosphate (Epox-PP)

Preparation of 3-methyl-3-butene-1-yl tosylate (isopentenyl tosylate):

2.32 mmol (442 mg) of tosyl chloride and 2.55 mmol (312 mg) of 4-(N,N-dimethylamino)pyridine are introduced while stirring with a magnetic stirrer into 5 ml of anhydrous dichloromethane in a glass reaction vessel which is equipped for handling under an inert atmosphere and has been carefully dried. 2.32 mmol (200 mg) of isopentenol dissolved in approx. 1 ml of dichloromethane are slowly added to this mixture through a septum using a syringe. The reaction is monitored by thin-layer chromatography on silica (silica gel 60 F-254; eluent: pentane/ethyl acetate 85/15 vol./vol.; R_f (product) = 0.4 and R_f (TsCl) = 0.5). After approx. 3 hours' stirring under a nitrogen atmosphere, the reaction mixture is diluted in a large volume of hexane (approx. 100 ml), resulting in the immediate formation of a white precipitate. The mixture is then filtered and the filtrate concentrated by evaporation under reduced pressure. The solution is then diluted with diethyl ether and refiltered. Once the solvent has evaporated, a yellowish oil is obtained. The product is purified by preparative chromatography through a silica column (silica gel 60; eluent: pentane/ethyl acetate 85/15). In this manner, 1.98 mmol (475 mg) of 3-methyl-3-butene-1-yl tosylate (85% isolated yield) are obtained. The compound (colorless oil) is stored at +4°C in an anhydrous medium.

Preparation of tris(tetra-n-butylammonium) hydrogenpyrophosphate:

4.5 mmol (1 g) of dihydrogenpyrophosphate disodium salt ($Na_2H_2P_2O_7$) are dissolved in 10 ml of cold deionized water which has previously been adjusted to pH 9 with a 10 mM ammonia solution. The solution is passed through a column containing 19 milliequivalents (4 g) of DOWEX® 50-WX8-200 cationic resin (H^+ form). The acid solution is eluted with 15-20 ml of cold deionized water at pH 9. The collected solution is immediately titrated at pH 7.3 using a 40% aqueous solution of tetra-n-butylammonium hydroxide (Bu_4NOH). After freeze-drying, 4 g of tetra-n-

butylammonium salt are obtained as a hygroscopic white solid. The salt is dissolved in 10 ml of anhydrous acetonitrile. The solution is subsequently filtered, then dried by evaporation of the solvent under reduced pressure in successive stages. In this manner, a solution of tris(tetra-n-butylammonium) hydrogenpyrophosphate is obtained with a purity of 98% (result deduced from analysis by ionic chromatography (HPAEC)). The volume is adjusted to achieve a salt concentration of between 0.5 and 1 M. The solution is stored at -20°C in an anhydrous medium.

Preparation of 3-methyl-3-butene-1-yl diphosphate (isopentenyl pyrophosphate):

2.5 ml of a solution of tris(tetra-n-butylammonium) hydrogenpyrophosphate at a concentration of 0.7 M (1.75 mmol) in anhydrous acetonitrile are introduced into a glass reactor which has been dried carefully. The reactor is cooled with an ice bath, then 0.70 mmol (168 mg) of 3-methyl-3-butene-1-yl tosylate dissolved in a minimum quantity of acetonitrile (0.5-1 M) are added using a syringe, while stirring with a magnetic stirrer. Once the tosylate has been introduced, the ice bath is removed, then the reaction is allowed to continue at room temperature with stirring. The progress of the reaction is then monitored by ionic chromatography (HPAEC). After approx. 3 hours, the solvent is evaporated under reduced pressure and the reaction medium redissolved in 3 ml of a 98/2 (vol./vol.) water/2-propanol mixture. The solution is passed through a column containing 19 milliequivalents (4 g) of DOWEX® 50-WX8-200 cationic resin (NH_4^+ form), then eluted with 10 ml of the water (pH 9)/2-propanol 98/2 (vol./vol.) mixture. After freeze-drying, a white solid containing the crude product is collected.

Purification:

The pyrophosphate and the traces of ammonium monophosphate are separated from the medium by coprecipitation in the presence of ammonium hydrogencarbonate. The crude product obtained in the preceding stage is dissolved in 4 ml of 0.1 M ammonium hydrogencarbonate, which is transferred into a 25 ml centrifuge tube. The solution is then treated with 10 ml of a 1/1 (vol./vol.) acetonitrile/2-propanol mixture by shaking the mixture vigorously (vortex) for a few minutes until a white precipitate has formed. The tube is then centrifuged at 2000 rpm at 10°C for 5 minutes. The supernatant, into which the organic salts have been extracted, is stored at +4°C. The

procedure is repeated by redissolving the precipitate in 3 ml of 0.1 M ammonium hydrogencarbonate, to which 7 ml of the acetonitrile/2-propanol mixture are added. The two supernatants are combined and the solvent evaporated under a vacuum. An oily liquid is obtained which is stored at +4°C.

The ammonium tosylate is separated from the reaction medium by extraction with the 1/1 (vol./vol.) chloroform/methanol solvent. The oily liquid from the preceding stage is dissolved in 4 ml of water at pH 9 and treated with 1 ml of this solvent by a conventional extraction procedure which is repeated 3 times. Any traces of solvent are then removed from the aqueous phase by evaporation under reduced pressure at 30°C. On the basis of analysis by ionic chromatography (HPAEC), an 83% yield of 3-methyl-3-butene-1-yl diphosphate (0.58 mmol, 172 mg) is obtained. The solution is stored at -20°C.

The product is then purified in accordance with requirements by anion exchange chromatography through 360 mg to 10 g Sep-Pak Accell Plus QMA (Waters®) cartridges eluted in succession respectively by 20 mM, 40 mM, 100 mM then 200 mM aqueous ammonium hydrogencarbonate solutions, with the eluted fractions being monitored by chromatography (HPAEC). The fractions corresponding to the purified product are combined and then freeze-dried.

Preparation of 3-(bromomethyl)-3-butanol-1-yl diphosphate:

0.34 mmol (100 mg) of isopentenyl pyrophosphate (ammonium salt) dissolved in 2 ml of neutral pH deionized water are treated under a fume hood at room temperature with 1.9 ml (0.34 mmol) of bromine in a saturated aqueous solution (0.18 M). The bromine solution is added gradually with the mixture being stirred periodically until the bromine water has become colorless. In the event that bromine is added in a slight excess (persistent yellow color), the solution is transferred into a glass flask and then exposed to reduced pressure (rotary evaporator) for a few minutes at a temperature of 30°C until the color disappears. The aqueous solution is filtered and then neutralized by being passed through a DOWEX® 50-WX8-200 cationic resin column (NH_4^+ form) eluted by two column volumes of deionized water. A solution of 3-(bromomethyl)-3-butanol-1-yl diphosphate (0.33 mmol, 130 mg) is obtained quantitatively and stored at -20°C.

Production of 3,4-epoxy-3-methyl-1-butyl diphosphate:

1 ml of the aqueous solution containing 3.35 mg (8.5 µmol) of 3-(bromomethyl)-3-butanol-1-yl diphosphate (ammonium salt) obtained in the preceding stage are treated at room temperature with 1 ml of a molar ammonia solution. The solution is stirred for a few minutes and then freeze-dried to remove the ammonia. The dry residue obtained after freeze-drying (2.7 mg, 8.5 µmol) is redissolved in 2 ml of deionized water. The bromide ions are removed from the solution using a DIONEX® apparatus comprising an OnGuard®-Ag cartridge attached to an OnGuard®-H cartridge (capacity 1.8 milliequivalents). The halide ions (bromides) are selectively retained as the solution passes through this apparatus, which is eluted with 1 ml of deionized water. For the purpose of performing biological testing, the aqueous solutions of the product are sterilized by filtration through a 0.2 µm filter and stored at -20°C. In the case of testing performed *in vivo*, the solutions are passed beforehand through a DOWEX® 50-WX8-200 cationic resin (Na^+ form) eluted by two column volumes of deionized water.

EXAMPLE 2: Production of 3,4-epoxy-3-methyl-1-butyl triphosphate (Epox-PPP):

Preparation of tetrakis(tetra-n-butylammonium) hydrogentriphosphate:

2.1 mmol (1 g) of tripolyphosphate hexahydrate pentasodium salt ($Na_5P_3O_{10}.6H_2O$) are dissolved in 10 ml of cold deionized water which has previously been adjusted to pH 9 with a 10 mM ammonia solution. The solution is passed through a column containing 21 milliequivalents (4.4 g) of DOWEX® 50-WX8 cationic resin (H^+ form). The acid solution is eluted with 20-25 ml of cold deionized water at pH 9. The collected solution is immediately titrated at pH 7.0 using a 40% aqueous solution of tetra-n-butylammonium hydroxide (Bu_4NOH). After freeze-drying, 2.5 g of tetra-n-butylammonium salt are obtained as a hygroscopic white solid. The salt is dissolved in 10 ml of anhydrous acetonitrile. The solution is subsequently filtered, then dried by evaporation of the solvent under reduced pressure in successive stages. In this manner, a solution of tetrakis(tetra-n-butylammonium) hydrogentriphosphate with a purity of 95% is obtained (result deduced from analysis by ionic chromatography (HPAEC)). The volume is adjusted to achieve a salt concentration of between 0.5 and 1 M. The solution is stored at -20°C in an anhydrous medium.

Preparation of 3-methyl-3-butene-1-yl triphosphate (isopentenyl triphosphate):

Using the procedure described for the preparation of 3-methyl-3-butene-1-yl diphosphate (Example 1), 2 mmol of a molar solution of tetrakis(tetra-n-

butylammonium) hydrogentriphosphate are reacted under a nitrogen atmosphere with 1 mmol (240 mg) of 3-methyl-3-butene-1-yl tosylate prepared according to Example 1 in 4 ml of anhydrous acetonitrile for 24 hours. By using a precipitation/extraction purification procedure similar to that applied to 3-methyl-3-butene-1-yl diphosphate, on the basis of analysis by ionic chromatography (HPAEC), a yield of 74% of 3-methyl-3-butene-1-yl triphosphate (0.74 mmol, 292 mg) is obtained. Phosphoepoxides according to the invention are prepared for the purpose of biological testing by using a fraction of the product obtained at this stage which is purified by HPAEC through an IonPac® AS11 column, with two or more chromatographic passes being combined. In this manner, approx. 2 ml of an aqueous millimolar solution of neutral pH of 3-methyl-3-butene-1-yl triphosphate are prepared in the form of an ammonium salt.

Preparation of 3-(bromomethyl)-3-butanol-1-yl triphosphate:

500 nmol (500 µl of a millimolar solution) of isopentenyl triphosphate are treated at room temperature by addition of 500 nmol of bromine in a saturated aqueous solution (2.8 µl of 180 mM bromine water). Once the mixture has been stirred and the bromine has become colorless (virtually instantaneously), the product 3-(bromomethyl)-3-butanol-1-yl triphosphate is produced quantitatively (0.5 ml of a millimolar solution).

Preparation of 3,4-epoxy-3-methyl-1-butyl triphosphate:

The solution obtained in the preceding stage containing 500 nmol of 3-(bromomethyl)-3-butanol-1-yl triphosphate is injected in several fractions into a Dionex® HPAEC system in accordance with "High pH anion exchange chromatographic analysis of phosphorylated compounds: application to isolation and characterization of non peptide mycobacterial antigens", Y. Poquet *et al*, Anal. Biochem, 243 no. 1, 1996, p. 119-126. The epoxide is formed quantitatively on each chromatographic pass and is collected in the presence of ammonium hydroxide or hydrogencarbonate. The collected fractions are freeze-dried. In this manner, approx. 1 ml of an aqueous 500 µM solution of 3,4-epoxy-3-methyl-1butyl triphosphate is obtained, which is treated as in Example 1 for the performance of biological testing and/or for the performance of *in vivo* testing and stored at -20°C.

EXAMPLE 3: Production of α,γ di-(3,4-epoxy-3-methyl-1-butyl) triphosphate (di EpoxTP):

Preparation of α,γ di-(3-methyl-3-butene-1-yl) triphosphate:

Using the procedure described for the preparation of 3-methyl-3-butene-1-yl diphosphate (Example 1), 0.5 mmol of a molar solution of tetrakis(tetra-n-butylammonium) hydrogentriphosphate (prepared according to Example 2) are reacted under a nitrogen atmosphere with 1 mmol (240 mg) of 3-methyl-butene-1-yl tosylate (prepared according to Example 1) in 4 ml of anhydrous acetonitrile for 24 hours. By using a precipitation/extraction purification procedure similar to that applied to 3-methyl-3-butene-1-yl diphosphate, on the basis of analysis by ionic chromatography (HPAEC), a yield of 81% of α,γ di-[3-methyl-3-butene-1-yl] triphosphate (0.4 mmol, 178 mg) is obtained. Phosphohalohydrin compounds according to the invention are prepared for the purpose of biological testing by using a fraction of the product obtained at this stage which is purified by HPAEC through an IonPac® AS11 column, with two or more chromatographic passes being combined. Before each chromatographic pass and in order to improve isolation of the product, the fraction to be purified is treated enzymatically with alkaline phosphatase in order to break down the isopentenyl triphosphate which is a secondary product of the reaction. In this manner, approx. 1 ml of an aqueous millimolar solution of neutral pH of α,γ di-[3-methyl-3-butene-1-yl] triphosphate are prepared in the form of an ammonium salt.

Preparation of α,γ di-[3-(bromomethyl)-3-butanol-1-yl] triphosphate:

250 nmol (250 µl of a millimolar solution) of α,γ di-(3-methyl-3-butene-1-yl) triphosphate are treated at room temperature by addition of 250 nmol of bromine in a saturated aqueous solution (1.4 µl of 180 mM bromine water). Once the mixture has been stirred and the bromine has become colorless (virtually instantaneously), the product α,γ di-[3-(bromomethyl)-3-butanol-1-yl] triphosphate is produced quantitatively (250 µl of a millimolar solution).

Production of α,γ di-(3,4-epoxy-3-methyl-1-butyl) triphosphate:

The solution obtained in the preceding stage containing 250 nmol of α,γ di-[3-(bromomethyl)-3-butanol-1-yl] triphosphate is injected in several fractions into a Dionex® DX500 HPAEC system as described in Example 2. In this manner,

approx. 0.5 ml of an aqueous 500 µM solution of α,γ di-[3,4-epoxy-3-methyl-1-butyl] triphosphate is obtained, which is treated as in Example 1 for the performance of biological testing and/or for the performance of *in vivo* testing and stored at - 20°C.

EXAMPLE 4: Production of uridine 5'-triphosphate γ-(3,4-epoxy-3-methyl-1-butyl) (Epox-UTP):

Preparation of uridine 5'-triphosphate γ-(3-methyl-3-butene-1-yl):

This product is prepared in accordance with the procedure described by KNORRE D. C. et al. "General Method for the synthesis of ATP Gamma-derivatives" Febs Letters, 1976, 70-1, 105-108, starting from 40 µmol of uridine 5'-triphosphate (UTP) (triethylammonium salt) in the presence of an excess of isopentenol. Phosphoepoxide compounds according to the invention are prepared for the purpose of biological testing by purifying a fraction of the product obtained by HPAEC through an IonPac® AS11 column, with two or more chromatographic passes being combined. Before each chromatographic stage and in order to improve isolation of the product, the fraction to be purified is treated enzymatically with alkaline phosphatase in order to break down the secondary products (UDP and UMP) and unreacted UTP. In this manner, approx. 500 µl of an aqueous 300 µM solution of neutral pH of uridine 5'-triphosphate γ-(3-methyl-3-butene-1-yl) are prepared in the form of an ammonium salt.

Preparation of uridine 5'-triphosphate γ-[3-(iodomethyl)-3-butanol-1-yl] :

75 nmol (250 µl of a 300 µM solution) of uridine 5'-triphosphate γ-3-methyl-3-butene-1-yl in ammonium salt form are treated in an aqueous medium of neutral pH by addition of 108 µl of 0.7 mM iodized water prepared according to Example 2. The solution is left for 20 minutes at room temperature while periodically being stirred vigorously. Once the iodized water has become colorless, the product uridine 5'-triphosphate γ-[3-(iodomethyl)-3-butanol-1-yl] is produced quantitatively (approx. 360 µl of a 200 µM solution).

Production of uridine 5'-triphosphate γ-(3,4-epoxy-3-methyl-1-butyl):

The solution obtained in the preceding stage containing 75 nmol of uridine 5'triphosphate γ-[3-(iodomethyl)-3-butanol-3-yl] is injected in several fractions into a Dionex® DX500 HPAEC system as described in Example 2. In this manner, approx. 0.5 ml of an aqueous 150 µM solution of uridine 5'-triphosphate γ-(3,4-epoxy-3-methyl-1-

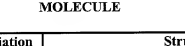
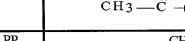
butyl) is obtained, which is treated as in Example 1 for the performance of biological testing and/or for the performance of *in vivo* testing and stored at - 20°C.

EXAMPLE 5: Measurement of antigenic activity by stimulation of the proliferation of Tγ9δ2 lymphocytes in a culture:

20 microliters of the aqueous solution of the compound according to the invention to be tested which has been adjusted to the final concentration specified in the test are added to an in vitro culture of 10^6 total T lymphocytes in 1 ml, separated from the blood of a healthy adult human donor and initially containing 1-5% of Tγ9δ2 lymphocytes, in an adequate culture medium (RPMI 1640+10% of inactivated human serum and 50 U/ml of human interleukin-2 (hIL-2)). After culturing for 4 days, 50 U of hIL-2 are added per milliliter of culture medium. After 8 days, the cells are counted, collected, washed with phosphate buffer, and the Tγ9δ2 type cells are detected in the culture by labeling with conventional commercial reagents (fluorescein-labeled monoclonal antibodies) and the proportion thereof determined by flux cytometry analysis. The parameter measured is either the change in the proportion of or the increase in the number of Tγ9δ2 cells in cultures in the presence of the compound according to the invention in comparison with cultures not containing a compound according to the invention. The results of this testing are represented by plotting curves of these values (y-axis in Figure 1) as a function of concentration on a logarithmic scale of the compound according to the invention placed in the culture (x-axis in Figure 1).

Figure 1 shows the results obtained with the compounds according to the invention obtained in Example 1 (Epox-PP), with the dotted line being a negative control (value obtained in the absence of the compound according to the invention).

Table 1 below shows the ED50 values, the effective dose at 50% of the maximum polyclonal lymphocyte amplification effect obtained as stated above with IPP (by way of comparison) and with various compounds according to the invention.

MOLECULE			ED 50% nM
Name	Abbreviation	Structure	
isopentenyl pyrophosphate	IPP		3000
3,4-epoxy-3-methyl-1-butyl diphosphate	Epox-PP		20
3,4-epoxy-3-methyl-1-butyl triphosphate	Epox-PPP		100
α, γ di 3,4-epoxy-3-methyl-1-butyl triphosphate	di-EpoxTP		2000

EXAMPLE 6: Measurement of antigenic activity by stimulation of induced cytotoxicity:

The specific cytotoxic activity of a Tγ9δ2 lymphocyte clone measured in accordance with the induced cytotoxicity test is compared, said activity being stimulated with decreasing concentrations of the phosphoantigen Tubag3 obtained as described by WO 95/20673 (curve represented by black squares in Figure 2), of the compound Epox-PP according to the invention obtained in Example 1 (curve represented by black circles in Figure 2), and of isopentenyl pyrophosphate (IPP, curve represented by black triangles in Figure 2).

It may be noted that the compound according to the invention from Example 1 is active at a concentration of the order of 20 nM, whereas the prior art compound IPP is active at a concentration of the order of 3 μM, or 150 times higher.

EXAMPLE 7: Demonstration of the difference in structure between an epoxide compound according to the invention and the natural mycobacterial Tubag phosphoantigens.

The bioactivity of the compound according to the invention Epox-PP (obtained as stated in Example 1) is compared with that of a stock preparation of Tubag natural phosphoantigens obtained as described in WO 95/20673.

Bioactivity is measured by an induced cytotoxicity test as described in Example 6 using the compound to be tested diluted 1:30 from 5 to 10 µM solutions of the compounds.

Before bioactivity is measured, the compounds are subjected to a transient chemical treatment by being brought into contact with one of the following reagents: $NaIO_4$ (5mM); $NaBH_3CN$ (10mM, pH 7); $KMnO_4$ (1mM); bromine water Br_2, H_2O (7mM), followed by neutralization of the reagent.

The following table shows the results obtained from several independent tests performed in each case. + indicates detection of activity upon Tγ9δ2 lymphocytes; - indicates that no activity could be detected.

	Bioactivity after chemical treatment:				
REAGENT	none	$NaIO_4$ *(5mM)*	$NaBH_3CN$ *(10mM pH7)*	$KMnO_4$ *(1mM)*	Br_2H_2O *(7mM)*
Tubag	+	+	+	-	-
Epox-PP	+	+	+	+	+

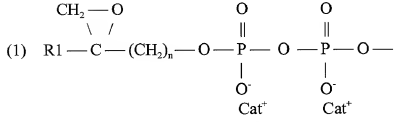
It may be noted that treating the natural mycobacterial Tubag phosphoantigens with dilute $KMnO_4$ or bromine water completely curtails the bioactivity thereof. In contrast, the phosphoepoxide compounds according to the invention, which have a stimulant action at concentrations of the order of 20 nM, resist the same chemical treatment, so demonstrating the difference in chemical structure between the synthetic compounds according to the invention and the natural Tubag compounds.

EXAMPLE 8: Toxicity of Epox-PP (sodium salt):

Five 30 g mice received an intravenous injection (caudal vein) of 300 µl of PBS buffer containing 1 mg of Epox-PP (sodium salt). No sign of shock or pyrogenicity is observed: 5 mice are still alive after 30 days; no significant variation in the weight of the mice is recorded during the study. Toxicity is thus less than 20% for a dose of 33.4mg of Epox-PP (sodium salt) per kilogram of animal body weight.

CLAIMS

1) Compounds comprising at least one phosphoepoxide group of the formula:



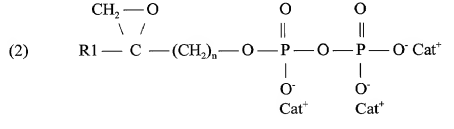
where R1 is selected from among —CH_3 and —CH_2—CH_3,

Cat+ is an organic or inorganic cation,

n is an integer between 2 and 20,

for the use thereof as therapeutically active substances.

2) Phosphoepoxide compounds of the formula:



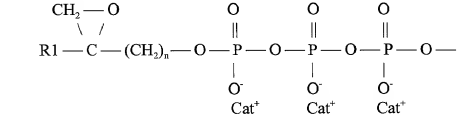
where R1 is selected from among —CH_3 and —CH_2—CH_3,

Cat^+ is an organic or inorganic cation,

n is an integer between 2 and 20,

for the use thereof as therapeutically active substances.

3) Novel compounds comprising at least one phosphoepoxide group of the formula:

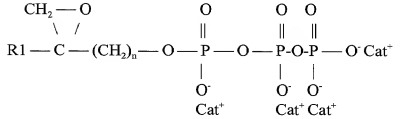


where R1 is selected from among —CH_3 and —CH_2—CH_3,

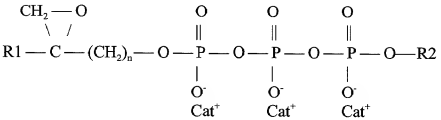
Cat^+ is an organic or inorganic cation,

n is an integer between 2 and 20.

4) Novel phosphoepoxide compounds as claimed in claim 3 of the formula:

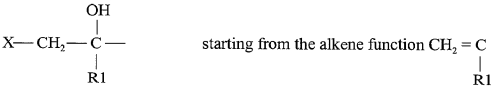


5) Novel phosphoepoxide compounds as claimed in claim 3 of the formula:



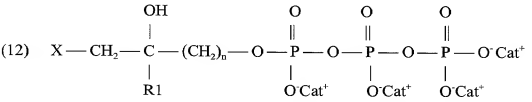
where R2 is an organic or inorganic substituent selected from among the group comprising:

- substituents which do not prevent formation of the halohydrin function

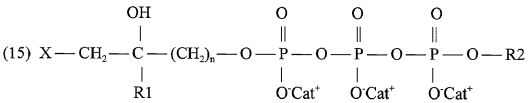


and halogen X_2 in the presence of water;

- and substituents for which there is an R2-O-Y compound which is not reactive towards the halohydrin function of the compound of the formula:



and selected such that R2-O-Y may react with the terminal phosphate of this compound (12) in order to obtain the compound (15):



- and substituents for which there is a compound R2-O-PPP, where PPP denotes the triphosphate group.

6) Compounds as claimed in one of claims 1, 3 and 5, comprising at least one group selected from among the group comprising nucleoside derivatives, oligonucleotides, nucleic acids (RNA, DNA), amino acids, peptides, proteins, monosaccharides, oligosaccharides, polysaccharides, fatty acids, simple lipids, complex lipids, folic acid, tetrahydrofolic acid, phosphoric acids, inositol, vitamins, co-enzymes, flavonoids, aldehydes, halohydrins and epoxides.

7) Compounds as claimed in claims 5 and 6, in which R2 is moreover selected from among the group comprising nucleoside derivatives, oligonucleotides, nucleic acids (RNA, DNA), amino acids, peptides, proteins, monosaccharides, oligosaccharides, polysaccharides, fatty acids, simple lipids, complex lipids, folic acid, tetrahydrofolic acid, phosphoric acids, inositol, vitamins, co-enzymes, flavonoids, aldehydes, halohydrins, phosphoepoxides of the formula (1) and epoxides.

8) Novel phosphoepoxide compounds of the formula:

(21)

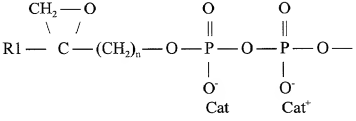
where R1 is selected from among —CH_3 and —CH_2—CH_3,
n is an integer between 2 and 20.

9) Compounds as claimed in one of claims 3 to 5, 7 or 8 for the use thereof as therapeutically active substances.

10) Compounds as claimed in one of claims 1 to 9 for the use thereof as Tγ9δ2 lymphocyte activators.

11) Compounds as claimed in one of claims 1 to 10 for the use thereof as Tγ9δ2 lymphocyte antigens in a therapeutic composition, in particular an immunostimulant therapeutic composition or a vaccine, for primates.

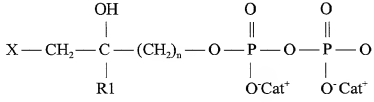
12) Process for the production of a compound comprising at least one phosphoepoxide group of the formula:



(1)

where R1 is selected from among $—CH_3$ and $—CH_2—CH_3$, Cat^+ is an organic or inorganic cation, n is an integer between 2 and 20, wherein:

- an intermediate compound comprising at least one phosphohalohydrin group of the formula is first prepared:

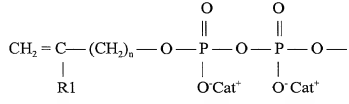


(6)

where X is a halogen selected from among I, Br, Cl,

- the intermediate compound is reacted with a hydroxide-producing medium in order to convert the halohydrin functions of the intermediate compound into epoxide functions.

13) Process as claimed in claim 12, wherein, in order to prepare said intermediate compound, the halogen X2 is reacted in the presence of water with a starting compound comprising at least one phosphorylated alkene group of the formula:



(7)

14) Process as claimed in one of claims 12 and 13, wherein the intermediate compound is reacted in a basic aqueous medium in order to convert the halohydrin functions of the intermediate compound into epoxide functions.

15) Composition for extracorporeal diagnostics, wherein it comprises at least one compound as claimed in one of claims 3 to 5, 7 or 8.

16) Therapeutic composition, wherein it comprises at least one compound as claimed in one of claims 1 to 8.

17) A therapeutic composition, wherein it comprises a quantity capable of being administered to a primate, in particular in contact with the peripheral bloodstream or topically, of at least one compound as claimed in one of claims 1 to 8.

18) The composition as claimed in one of claims 15 to 17, wherein it moreover comprises primate Tγ9δ2 lymphocytes.

19) The composition as claimed in one of claims 15 to 18, wherein it moreover comprises a proportion of interleukin suitable to bring about lymphocyte growth in the medium into which it is to be administered.

20) A process for the production of a composition having the characteristic of activating Tγ9δ2 lymphocytes, in which at least one compound as claimed in one of claims 1 to 8 is used.

21) A process for the production of a therapeutic composition intended for the preventive or curative treatment of a pathological condition which produces cells sensitive to Tγ9δ2 lymphocytes, in which process at least one compound as claimed in one of claims 1 to 8 is used.

22) A process for the production of a therapeutic composition intended to be administered to a primate for the preventive or curative treatment of a pathological condition which produces cells sensitive to Tγ9δ2 lymphocytes, in which process at least one compound as claimed in one of claims 1 to 8 is used.

23) A process for the production of a therapeutic composition intended to be administered to a primate for the preventive or curative treatment of a pathological condition selected from among the group comprising cancers, infectious diseases, parasitic conditions, and pathological immunodeficiency syndromes, in which process at least one compound as claimed in one of claims 1 to 8 is used.

24) The process according to claim one of claims 20 to 23, in which at least one compound as claimed in one of claims 1 to 11 is brought into contact with a medium which contains Tγ9δ2 lymphocytes, and is compatible with T lymphocyte growth, in a quantity suitable for activating these Tγ9δ2 lymphocytes in this medium.

25) The process as claimed in claim 24, in which said medium comprises a substance selected from among primate blood and primate blood extracts.

26) An extracorporeal Tγ26δ9 lymphocyte activation process, in which the Tγ9δ2 lymphocytes are brought into contact with at least one compound as claimed in one of claims 1 to 8 in an extracorporeal medium which contains Tγ9δ2 lymphocytes and is compatible with T lymphocyte growth.

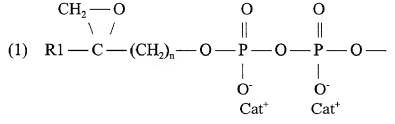
27) The process as claimed in claim 26, in which at least one compound as claimed in one of claims 1 to 8 is used at a concentration in the medium which brings about activation of polyclonal proliferation of Tγ9δ2 lymphocytes.

28) The process as claimed in one of claims 26 to 27, in which a proportion of interleukin suitable to bring about lymphocyte growth in the medium is introduced into the medium.

ABSTRACT

PHOSPHOEPOXIDES, PROCESS FOR THE PRODUCTION THEREOF AND USE THEREOF.

The invention provides compounds comprising at least one phosphoepoxide group of the formula:



where R1 is selected from among $-CH_3$ and $-CH_2-CH_3$,

Cat+ is an organic or inorganic cation,

n is an integer between 2 and 20,

processes for the production thereof and uses thereof, in particular therapeutic uses and for activating primate Tγ9δ2 lymphocytes.

Fig. 1

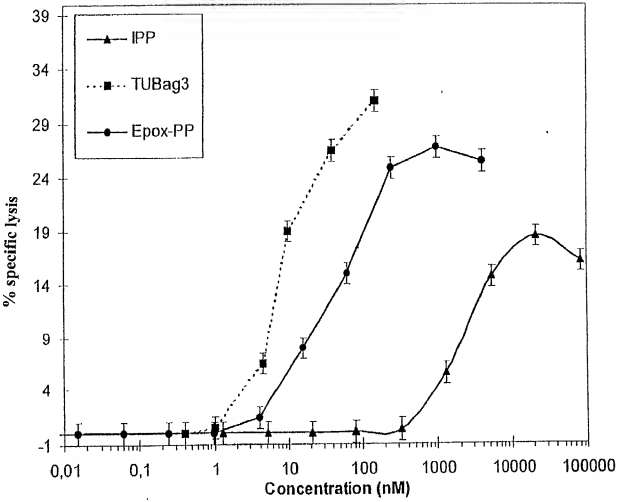


Fig. 2

Declaration and Power of Attorney for Patent Application

Déclaration et Pouvoirs pour Demande de Brevet

French Language Declaration

En tant que l'inventeur nommé ci-après, je déclare par le présent acte que:

As a below named inventor, I hereby declare that:

Mon domicile, mon adresse postale et ma nationalité sont ceux figurant ci-dessous à côté de mon nom.

My residence, post office address and citizenship are as stated next to my name.

Je crois être le premier inventeur original et unique (si un seul nom est mentionné ci-dessous), ou l'un des premiers co-inventeurs originaux (si plusieurs noms sont mentionnés ci-dessous) de l'objet revendiqué, pour lequel une demande de brevet a été déposée concernant l'invention intitulée

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

PHOSPHOEPOXYDES, PROCEDE DE FABRICATION

ET APPLICATIONS

et dont la description est fournie ci-joint à moins que la case suivante n'ait été cochée:

the specification of which is attached hereto unless the following box is checked:

☒ a été déposée le 27 août 1999
sous le numéro de demande des Etats-Unis ou le numéro de demande international PCT PCT/FR 99/02057 et modifiée le _____ (le cas échéant).

☐ was filed on _____
as United States Application Number or PCT International Application Number _____ and was amended on _____ (if applicable).

Je déclare par le présent acte avoir passé en revue et compris le contenu de la description ci-dessus, revendications comprises, telles que modifiées par toute modification dont il aura été fait référence ci-dessus.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

Je reconnais devoir divulguer toute information pertinente à la brevetabilité, comme défini dans le Titre 37, § 1.56 du Code fédéral des réglementations.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

French Language Declaration

Je revendique par le présent acte avoir la priorité étrangère, en vertu du Titre 35, § 119(a)-(d) ou § 365(b) du Code des Etats-Unis, sur toute demande étrangère de brevet ou certificat d'inventeur ou, en vertu du Titre 35, § 365(a) du même Code, sur toute demande internationale PCT désignant au moins un pays autre que les Etats-Unis et figurant ci-dessous et, en cochant la case, j'ai aussi indiqué ci-dessous toute demande étrangère de brevet, tout certificat d'inventeur ou toute demande internationale PCT ayant une date de dépôt précédant celle de la demande à propos de laquelle une priorité est revendiquée.

I hereby claim foreign priority under Title 35, United States Code, § 119(a)-(d) or § 365 (b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below, and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior foreign applications

Demande(s) de brevet antérieure(s) dans un autre pays:

(Number) (Numéro)	(Country) (Pays)	(Day/Month/Year Filed) (Jour/Mois/Année de dépôt)	Priority claimed / Droit de priorité revendiqué: Yes / Oui	No / Non
98.10914	FRANCE	1st September 1998	☒	☐
			☐	☐
			☐	☐

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35, § 119(e) du Code des Etats-Unis, de toute demande de brevet provisoire effectuée aux Etats-Unis et figurant ci-dessous.

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

(Application No.) (N° de demande)	(Filing Date) (Date de dépôt)

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35, § 120 du Code des Etats-Unis, de toute demande de brevet effectuée aux Etats-Unis, ou en vertu du Titre 35, § 365(c) du même Code, de toute demande internationale PCT désignant les Etats-Unis et figurant ci-dessous et, dans la mesure où l'objet de chacune des revendications de cette demande de brevet n'est pas divulgué dans la demande antérieure américaine ou internationale PCT, en vertu des dispositions du premier paragraphe du Titre 35, § 112 du Code des Etats-Unis, je reconnais devoir divulguer toute information pertinente à la brevetabilité, comme défini dans le Titre 37, § 1.56 du Code fédéral des réglementations, dont j'ai pu disposer entre la date de dépôt de la demande antérieure et la date de dépôt de la demande nationale ou internationale PCT de la présente demande:

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

(Application No.) (N° de demande)	(Filing Date) (Date de dépôt)	(Status) (patented, pending, abandoned) (Statut) (breveté, en cours d'examen, abandonné)

Je déclare par le présent acte que toute déclaration ci-incluse est, à ma connaissance, véridique et que toute déclaration formulée à partir de renseignements ou de suppositions est tenue pour véridique; et de plus, que toutes ces déclarations ont été formulées en sachant que toute fausse déclaration volontaire ou son équivalent est passible d'une amende ou d'une incarcération, ou des deux, en vertu de la Section 1001 du Titre 18 du Code des Etats-Unis, et que de telles déclarations volontairement fausses risquent de compromettre la validité de la demande de brevet ou du brevet délivré à partir de celle-ci.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

French Language Declaration

POUVOIRS: En tant que l'inventeur cité, je désigne par la présente l'(les) avocat(s) et/ou agent(s) suivant(s) pour qu'ils poursuive(nt) la procédure de cette demande de brevet et traite(nt) toute affaire s'y rapportant avec l'Office des brevets et des marques: *(mentionner le nom et le numéro d'enregistrement).*

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: *(list name and registration number)*

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ROBERT J. PATCH, 703/521-2297

Nom complet du seul ou premier inventeur Christian BELMANT	Full name of sole or first inventor
Signature de l'inventeur Date 15/01/2001	Inventor's signature Date
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Signature de l'inventeur Date 15.1.2001	Second Inventor's signature Date
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(Fournir les mêmes renseignements et la signature de tout co-inventeur supplémentaire.)

(Supply similar information and signature for third and subsequent joint inventors.)

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Signature de l'inventeur Date 22.01.01	Inventor's signature Date
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Signature de l'inventeur Date 24.1.01	Second inventor's signature Date
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Nom complet du cinquième co-inventeur, le cas échéant	Full name of sole or fifth joint inventor, if any
Signature de l'inventeur Date	Inventor's signature Date
Domicile	Residence
Nationalité	Citizenship
Adresse Postale	Post Office Address
Nom complet du sixième co-inventeur, le cas échéant	Full name of sixth joint inventor, if any
Signature de l'inventeur Date	Second Inventor's signature Date
Domicile	Residence
Nationalité	Citizenship
Adresse Postale	Post Office Address